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BRUCE FINK, LICHENOLOGIST

ROBERT B. WYLIE

(WITH PLATE 1)

Dr. Bruce Fink, foremost lichenologist in this country, died July 10, 1927. Following a usual custom, he had gone to his laboratory early in the morning and an assistant there found him with his head resting on his arms, death having resulted from heart failure.

Born at Blackberry, Ill., Dec. 22, 1861, and obtaining degrees from Illinois, Harvard and Minnesota, Dr. Fink has been closely identified with the botanical work of this country for over thirty years. Beginning his teaching work at Upper Iowa University in 1892, he was made professor of botany at Grinnell College in 1903, and in 1906 accepted the headship of Botany at Miami University, which position he held until the end of his life.

Dr. Fink's research was mainly in the field of lichenology, and his activity as a scientist is attested by over one hundred titles, covering published notes, reviews, papers and monographs. Primarily a taxonomist, he contributed also to the biology of lichens with discussions of their classification, relationships and distribution. He had in preparation a manual of the lichens of the United States. Numerous papers dealt with fungi and particularly with the Ascomycetes. His botanical interests were broad and he followed with zest the marked development of the plant sciences as a whole which occurred during his scientific lifetime. Will any other period witness greater development and

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diversification than marked plant study and research from 1892 to 1927?

As a teacher, Professor Fink always carried a heavy class load and was greeted each year by large numbers of students. An unusual percentage of these were inspired to take advanced work in botany, and many of his students entered scientific work professionally.

Dr. Fink will be remembered by all who knew him as a man of genial personality and optimistic temperament. These qualities in combination with his modesty and generosity made him the friend of all classes. Of simple tastes, he loves his home and his laboratory and through these revealed a life of marked sincerity and high achievement.

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THE SEXUALITY AND ARRANGEMENT OF THE SPORES IN THE ASCUS OF NEUROSPORA SITOPHILA

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(WITH PLATE 2 AND 2 TEXT FIGURES)

Although considerable work has been done on the cytology of heterothallism in the Basidiomycetes, heretofore no one has investigated the sexual nature of particular spores in the asci of heterothallic Ascomycetes. Newton (**23**) determined experimentally the nature of the four spores as to their position on the basidium of *Coprinus Lagopus*. Betts (**3**) found that of seven single spore cultures from an ascus of *Ascobolus carbonarius* three were of one haplont and four of the other. Shear and Dodge (**26**) report that for the five different asci of *Neurospora crassa* examined four of the spores are of one sex and four of the other sex.

At a recent meeting of the Botanical Society of Washington, Dr. B. O. Dodge discussed the cytological basis for heterothallism in species of the *Monilia sitophila* group. He showed that while segregation of the factors for sexuality probably takes place in the first division in the four-spored homothallic species, *Neurospora tetrasperma* (**26**), it could take place in either of the other two divisions with the same effect. As a result of his studies he thought, however, that the differentiation of the sex character in the eight-spored heterothallic species *N. sitophila* (**26**) probably takes place in the second division, and that the spores in the ascus would alternate, two of one sex and two of the other. At his suggestion, and under his direction, the writer has undertaken to determine by means of cultures and a study of nuclear behavior the point of segregation of the sex factors in *N. sitophila*, and the sexual nature of each of the eight spores according to its position in the ascus.

In the basidium the orientation of the spindles during the mitoses and the position of the four daughter nuclei might not, owing to the particular features of the basidium, be any criterion

TABLE I

Fungus	Author	1st mitosis	2d mitosis	3d mitosis
Peziza vesiculosa	Gjurasin (12)	long.	long. or obl.	trans.
Peziza Stevensoniana	Harper (14)	long.	long.	
Lachnea scutellata	Harper (15)	long.	long. or obl.	trans. or long.
Pyronema confluens	Harper (16)	trans.	long. or conj.	trans.*
Galactinia succosa	Maire (22)	long.	long.	trans. or obl.
Morchella esculenta	Maire (22)	obl.	long.* or obl.	trans or. obl.
Pustularia vesiculosa	Maire (22)	long.	long.	trans. or obl.
Rhytisma acerinum	Maire (22)	long.	———	trans.* or obl.
Hydnobolites sp.	Faull (7)	eccentric	peripheral	parallel
Neotiella albocincta	Faull (7)	variable	variable	variable
Sordaria fimicola	Faull (7)	long.*	long.*	trans. or obl.
Phyllactinia corylea	Harper (17)	long.	variable	variable
Humaria rutilans	Fraser (9)	long.	obl.	trans. or obl.
Otidea aurantia	Fraser & Welsford (11)	long. or obl.	long.	variable
Peziza vesiculosa	Fraser & Welsford (11)	long.	long.	trans. or obl.
Ascobolus furfuraceus	Fraser & Brooks (10)	long. or obl.	long.	trans. or obl.
Humaria granulata	Fraser & Brooks (10)	———	trans.	trans.
Lachnea stercorea	Fraser & Brooks (10)	obl.	trans. or obl.	trans.
Geoglossum glabrum	Jolivette (18)	long.	long.*	obl.
Galactinia succosa	Guilliermond (13)	long.	long.	variable
Pustularia vesiculosa	Guilliermond (13)	———	long.	trans.
Pyronema confluens	Claussen (4)	variable	variable	trans. or obl.
Laboulbenia chaetophora	Faull (8)	long.	long.	long.
Collema pulposum	Bachmann (1)	long.	———	long.
Verpa bohemica	Komarnitzky (20)	long.	trans. or obl.	trans.
Ascobolus immersus	Ramlow (24)	trans.	trans. or obl.	long.
Philocopra coeruleotecta	Sax (25)	trans.	———	long.
Pustularia bolarioides	Bagchee (2)	long.	long. or obl.	trans. or obl.
Ophiobolus graminis	Jones (19)	long.	long. obl. or conj.	variable
Neurospora tetrasperma	Dodge (6)	long.	long. obl. or conj.	trans. or obl.

* Not figured, but orientation described in text.

for determination of the sexual nature of each of the four spores. There can be, however, no question that in the heterothallic Ascomycetes, especially those with long slender asci, the position of the daughter nuclei and orientation of spindles in the three mitoses do furnish, when taken in connection with the results of culture studies, a very reliable basis for determining when segregation of sex factors takes place.

As regards its orientation with respect to the long axis of the ascus, a spindle has been referred to as longitudinal, transverse or oblique. When the orientation is in any plane from longitudinal to transverse, the positions may be described as variable. Divisions in an ascus in which the spindles extend in the same direction and lie close together are referred to as conjugate, without implying that they serve the purpose usually attributed to conjugate division in the rusts. In table I are brought together chronologically statements of previous authors bearing on the position and orientation in the divisions of the nuclei of the asci in various species of Ascomycetes.

It will be seen from the table that in comparatively long asci the spindles of the first division are most frequently longitudinally orientated. The spindles of the second mitosis are also longitudinal, but less constantly so; while the spindles of the third mitosis are commonly transversely placed. Several exceptions in each case occur, however, which may or may not have some significance in connection with the segregation of particular factors.

Several investigators have worked out the cytology of the Basidiomycetes and find the spindles also variously placed. Levine (**21**), for example, states that the long axis of the spindles in both divisions is commonly transverse to the long axis of the basidium. The spindles may also lie in a position parallel to the long axis of the basidium.

CYTOLOGY OF THE ASCUS OF *Neurospora sitophila*

The material for cytological work was obtained from cultures made by inoculating corn meal agar test tubes with mycelia or conidia of the two reciprocal haplonts, *A* and *B* (**26**). In a week or ten days small pieces of agar-bearing perithecia were fixed in

Flemming's weaker solution. Sections were cut five microns thick and stained with the triple stain.

The ascus of *Neurospora sitophila* is long and narrow, such as is figured by Fraser & Welsford (11) for *Peziza vesiculosa*. The spindle of the first division lies in the long axis of the ascus, or nearly so, as is quite regularly the case in such long narrow asci.

After the first division is completed the reorganized daughter nuclei move some distance apart (PLATE 2, *A*). This drawing apart of the daughter nuclei is figured by Harper (17) in *Phyllactinia* and in *Peziza vesiculosa* by Fraser and Welsford (11). The second mitosis then takes place and the spindles are correspondingly some distance apart. They lie in the long axis of the ascus (PLATE 2, *B*).

The four resulting nuclei are somewhat bottle-shaped and have two long narrow antler-like structures extending from the neck (PLATE 2, *C*). The two nuclei in each half of the ascus are sister nuclei, symmetrically oriented. In every section in which this stage was found the horns of the first and third nuclei point upward while those of the second and fourth point downward. Aside from similar appendages described by Dodge (6) for *Neurospora tetrasperma* the writer has found no reference to anything resembling such structures in the literature on the fungi. In *N. sitophila* they are somewhat longer and more definitely developed organs than are those of *N. tetrasperma*. In the latter species, according to Dodge, frequently after the second division there must occur such a change of position of the pair of daughter nuclei as to insure that two non-sister nuclei come to lie in each end of the ascus. No such shifting occurs in *N. sitophila*.

The spindles of the third division are transverse and are placed some distance apart. At this stage the spore plasm of the ascus is divided into four parts, the regions lying between the spindles being filled with large vacuoles (PLATE 2, *D*). This would seem to preclude the possibility of any material change in the position either of the spindles during the third mitosis or of the pairs of daughter nuclei resulting from this division.

The stages between reorganization of the eight nuclei and spore delimitation are not figured. The astral rays do not show very

distinctly in preparations. Each nucleus is, however, drawn out into a long curved beak from which develop very fine rays which are to take part in cutting out of spores. Opposite the nuclear beak in each spore (PLATE 2, *E*) are developed numerous large vacuoles. The processes are essentially the same as described for the small spores of *N. tetrasperma* (**6**) where only one nucleus is included in each spore. Normally in *N. tetrasperma* each ascus contains four spores, two of the eight nuclei in the ascus being included in each spore. At spore delimitation these two nuclei lie side by side and coöperate in the process.

Shear and Dodge (**26**) have shown that each normal spore contains all the elements necessary for sexual reproduction so that the spore is ordinarily homothallic. In a later paper Dodge (**6**) has shown that when an ascus has more than four spores, the small spores are not totipotent and contain only one nucleus. Sexual reproduction can only be brought about in such cases by mating reciprocal haplonts obtained by germinating the small spores. In the normal or two-nucleate spores the nuclei are non-sisters, being developed at similar poles of adjacent spindles of the third division.

N. sitophila is heterothallic. Culture work to be described later in this paper shows that of the spores in one end of the ascus the upper two are of one sex and the next two of the opposite sex. The cytology of the ascus of this species just described shows how this comes about. The two spores uppermost in the ascus, for example, must contain nuclei which have developed at the poles of the upper spindle. The evidence presented all goes to show that segregation of the sex factors can not occur in either the third or first divisions. If segregation were to take place in the third division, the spores would alternate in the ascus, male and female. If segregation took place in the first division when reduction is presumed to occur, then all four spores in one end of the ascus would be of the same sex, which is certainly not the case in *N. sitophila*.

In such long and narrow asci there is little possibility of spores shifting positions with reference to each other, as might occur in a broad ascus of the type of *N. tetrasperma* or of powdery mildews. Still, there can be no question that the spores do readjust them-

selves by rotating as they elongate after being fully delimited. This is clearly shown in plate 2, *E*, where the nuclei of the upper four spores all point practically in the same direction while the nuclei of the lower four spores tend to point in the opposite direction. The careful study of two sections of this ascus indicates that the beaks of the nuclei are in reality arranged along a half spiral.

A later stage in the delimitation of the spores showing retraction of the nucleus is figured (PLATE 2, *F*). In this particular ascus all the spores were pointed the same way, showing clearly that considerable movement and twisting of the spores occur after they are cut out. The curved end of the beak which stains heavily still shows plainly long after the nucleus has become detached. While each spore is at first uninucleate, a further division takes place similar to those figured by Maire (**22**) in *Morchella*, Faull (**7**) in *Sordaria*, and Dodge (**6**) in *Neurospora tetrasperma*, so that each spore of *N. sitophila* at maturity contains two sister nuclei (PLATE 2, *H*).

CULTURE STUDIES

Ascospore material for this study of *Neurospora sitophila* originally came from Arlington, Va., and Japan (**26**). The perithecia used were grown in culture made by transferring to corn meal agar bits of mycelia or conidia from cultures having mycelia of both sexes. It was found that the spores would not germinate if isolated from the ascus before they have become fully colored. If, on the other hand, the ascus is too old, the wall is apt to burst before its spores can be isolated in order.

The point of an ordinary dissecting needle was too large for isolating the spores from the ascus, although an expensive micromanipulator is not at all essential. The finest sewing needles that could be purchased were found to be quite satisfactory.

Five or six perithecia which are just beginning to discharge their first spores were crushed out in a drop of sterile water on a slide. After a number of mature asci had been separated from the crushed masses they were transferred with a part of the water to 4 per cent corn meal agar in petri dishes. Using a binocular microscope and a fine pointed needle, individual asci were isolated

on the surface of this hard agar. The spores could then be removed one by one from the ascus and pushed a few millimeters away where their relative positions in the ascus were recorded by scratching the number on the agar.

These ascospores do not ordinarily germinate at room temperature (**26**). In this they are like spores of certain species of *Ascobolus* which must be heated before they will germinate. The method described by Dodge (**5**) has been found adequate for this work. Since *Neurospora sitophila* produces conidia in abundance, special precautions must be taken to avoid contamination of the single ascospore cultures. After isolating the ascospores it is advisable to allow the petri dish to stand for several hours so the conidia may begin to germinate. The germinating conidia can then be killed by the heating necessary to germinate the ascospores (**26**).

The plates containing the isolated ascospores were placed in an oven at room temperature and slowly raised to about 90° C. temperature. This usually took from fifteen to twenty minutes. In from five to twelve hours after heating, depending on temperature of room where cultures were kept, some spores were found to have germinated. Each germinating spore was then carefully removed on a bit of agar and transferred directly to corn meal agar in a test tube.

In about three days or as soon as enough conidia had formed in these cultures, masses of conidia of each haplont were transferred to separate tubes of melted agar. To avoid possibility of contamination or mixture of spores of the various haplonts, different culture rooms were used for making the transfers. After the agar containing spore suspensions had hardened, transfers could be made without the danger of contaminations such as would occur if dry conidia were transferred directly. All possible combinations of the haplonts from each ascus were then made. Perithecia usually begin to develop within eight or ten days, but as they at first resemble bodies which soon appear in the tubes containing single haplonts, it is necessary to wait two or three weeks before results can be determined definitely.

As might be expected, in the majority of the trials the writer failed to obtain germination of all eight spores in the ascus.

Unlike the spores of *Ascobolus carbonarius* which will germinate before they are mature and while still colorless or half grown (5) the spores of *Neurospora sitophila* must reach full maturity before germination will occur.

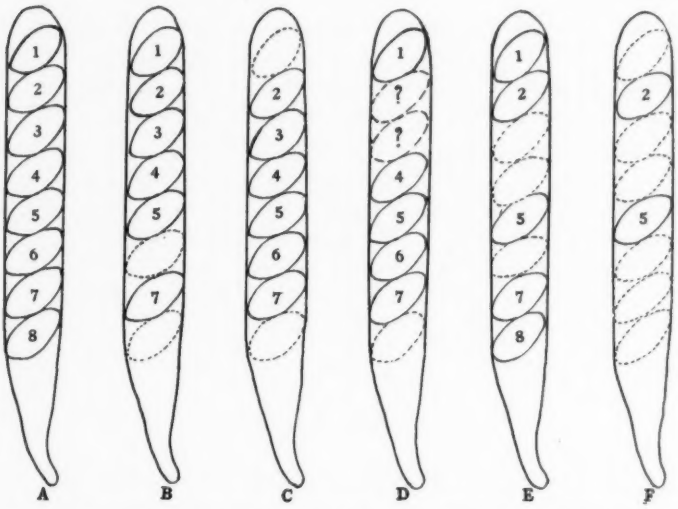

FIG. 1. Diagram of asci showing number and position of spores germinated after isolating from ascus. Germinated spores are shown in solid line

In only one instance was full germination obtained, but in five other cases the position of the spores that did germinate was such that it could be determined with practical certainty what the sexuality of the missing spores originally was. The positions of the spores from which haplont mycelia were obtained are shown in text figure 1. The spores are numbered from the tip of the ascus downward.

As noted above, in only one instance (*A*) did all eight of the spores germinate. In three cases (*B*, *C*, *D*) six of the spores germinated; in one case (*E*) five spores developed mycelia and in one trial (*F*) only two spores grew. At this point it may be stated that the terms "sex" and "sexuality" are used without knowing whether or not a monosporous mycelium of *N. sitophila* produces functional oögonia or antheridia. A monosporous

mycelium is referred to as "haplont." The results of mating the eight different haplonts obtained by germinating the eight spores in the ascus represented by *A* in text figure 1 are given in table II. Failure to produce perithecia is indicated by a minus sign (−), a positive sexual reaction resulting in the formation of normal perithecia is indicated by the plus sign (+).

TABLE II

RESULTS OF GROWING TOGETHER IN DUPLICATE IN ALL POSSIBLE COMBINATIONS THE EIGHT HAPLONT MYCELIA OBTAINED BY GERMINATING THE EIGHT ASCOSPORES FROM A SINGLE ASCUS OF *Neurospora sitophila*

	1	2	3	4	5	6	7	8
1.	−	−	+	+	+	+	−	−
2.	−	−	+	+	+	+	−	−
3.	+	+	−	−	−	−	+	+
4.	+	+	−	−	−	−	+	+
5.	+	+	−	−	−	−	+	+
6.	+	+	−	−	−	−	+	+
7.	−	−	+	+	+	+	−	−
8.	−	−	+	+	+	+	−	−

Mycelia from spores Nos. 1, 2, 7 and 8 in this ascus produce no perithecia when grown in combination with each other. They must, therefore, have like sex factors. Mycelia from spores Nos. 3, 4, 5 and 6 likewise produce negative results when grown with each other and are, then, like haplonts as to their sexuality. It will be seen, however, that when the haplont from spore No. 1, 2, 7 or 8 is grown with the haplont from spore No. 3, 4, 5 or 6, a positive sexual reaction occurs which is indicated by the formation of perithecia.

The particular sex of the haplont in no case has as yet been determined. Shear and Dodge (26) have referred to haplonts of this species which were reciprocal as to their sexuality merely as haplonts *A* and *B*. The writer has determined the sexuality of the eight haplonts under discussion by mating haplonts No. 2 and 5 with the known haplonts *A* and *B* obtained from those authors.

The results obtained show the spores 1, 2, 7 and 8 contain what for convenience may be called the *B* factors for sexuality while spores 3, 4, 5 and 6 contain the *A* factors for sexuality.

These results are shown diagrammatically in text figure 2, where the spores which developed haplont *A* are shown in white and haplont *B* in black.

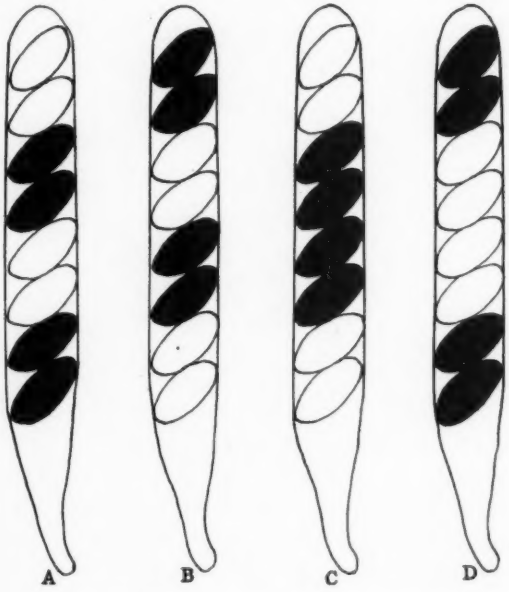

FIG. 2. Diagram showing the four possible arrangements of spores in the ascus according to sex as determined by cultural results. White spores are haplont A, blackened spores are haplont B.

In ascus *B*, text figure 1, the 6th and 8th spores did not germinate. Judging from what is known from the writer's cytological studies of *N. sitophila* previously discussed in this paper, and from the results obtained by the pairing of like haplonts as has just been shown where the sex nature of each spore in the ascus was determined experimentally, spore No. 6 should carry the same sex factors as spore No. 5 and spore No. 8 should bear the same factors for sexuality as spore No. 7. The results obtained after mating spores from this ascus with known haplonts *A* and *B* (**26**) are shown in text figure 2-*A*. In this ascus the sexes of the spores alternate in pairs, the first pair carrying the *A* sex factors, the second pair carrying the *B* sex factors, and so on.

In trial No. 3 (TEXT FIG. 1-*C*) spores Nos. 1 and 8 were missing. On the basis discussed above one can assume that spore No. 1 is like spore No. 2, and that spore No. 8 is like spore No. 7. Text figure 2-*B* will then represent the picture showing the results actually obtained by growing six of the eight haplonts in all possible combinations as completed by the addition of the missing haplonts, Nos. 1 and 8. Here, too, the sexes of the spores alternate in pairs, the first and third pairs in this instance carrying the *B* sex factors and the second and fourth pairs bearing the *A* factors for sexuality, the reverse of the condition represented in text figure 2-*A*.

In ascus "*D*" (TEXT FIG. 1) spores 1, 3(?), 4, 5, 6 and 7 developed mycelia. The results of the combinations made show that spores 1, 5 and 6 carried the *A* factors for sexuality and spores 4 and 7 the *B* sex factors. In isolating the spores from this ascus the writer was not positive of the order of the 2d and 3d spores, and this was borne out by the results of the culture. According to the results of the pairing, the haplonts in the upper end of the ascus alternated, and it is assumed that the spore originally labeled No. 3 was in reality the second spore in the ascus. As previously discussed, all evidence goes to show that like haplonts arrange themselves in pairs along the length of the ascus. Supplying the missing spores on that basis, the picture figure 2-*A*, formed by the arrangement of the spores in this ascus, is the same as that for ascus *B*, discussed previously. In general there is no tendency to shift positions after the spore has been delimited. The fact that the ascus is long and narrow and the third division spindles are placed far apart and transversely oriented is an assurance against any shifting or displacement of the spores. If, however, the eight spores of a large number of asci were germinated, it would be indeed strange if an exception to the general order would not be found, so that an alternation of sexes in one end of the ascus might result.

The results of making all possible combinations in culture of the five haplonts which were obtained by germinating the spores indicated in text figure 1-*E* forms the picture *B* of text figure 2 as completed by inserting the missing haplonts on the basis discussed above. The arrangement of the spore haplonts in

this ascus is the same as that of ascus *C* (TEXT FIG. 1). Spores 1, 2 and 5 are haplont *B* and spores 7 and 8 haplont *A*.

It might be thought that when only two spores from an ascus germinate, such results should be disregarded as of no consequence. Even such meager results are helpful in arriving at a conclusion as to the constancy of the location in the ascus of "male" and "female" spores. In ascus "*F*" (TEXT FIG. 1) only the second and fifth spores germinated, and when combined with each other and with known haplonts, spore No. 2 proved to be haplont *A* and spore No. 5 haplont *B*. From what is known of the pairing of haplonts, it is assumed that spore No. 1 bears the same sex factors as spore No. 2, namely "*A*," and that spore No. 6, like spore No. 5, carries the "*B*" factors for sexuality. The evidence afforded by a knowledge of the nuclear behavior in the ascus, supplemented by the results of the culture work just presented, supports the claim previously set forth (p. 7) that segregation of sex factors in this species must take place in the second division in the ascus. Therefore, if the first two spores of this ascus are haplonts *A*, spores 3 and 4 must be of the opposite sex, or haplonts *B*. If spores 5 and 6 are haplonts *B*, the spores 7 and 8 must contain the *A* sex factors, making the picture shown in "*C*" of text figure 2.

Of the six asci of *N. sitophila* described above the spore haplonts in two of the asci form the picture *AA*, *BB*, *AA*, *BB*; two asci form the picture *BB*, *AA*, *BB*, *AA*; and one ascus each makes the picture *AA*, *BB*, *BB*, *AA* and *BB*, *AA*, *AA*, *BB*, respectively. In every case it will be seen the spores arrange themselves in pairs with both sexes in each end of the ascus. This is explained by a study of the cytology of the species. The ascus is long and narrow and the spindle of the first mitosis is longitudinally placed. The resulting daughter nuclei move some distance apart and the spindles of the second division are also longitudinal. The four resting nuclei are distributed at equal distances along the length of the ascus and, upon dividing, the spindles are transversely oriented. Between the spindles the sporeplasm is filled with large vacuoles, which would tend to prevent any material change in the position either of the spindles of the third division or of the pairs of resulting daughter nuclei.

The two upper spores, then, must contain the nuclei which developed at the poles of the upper spindle and are, therefore, sister nuclei, the spores carrying like factors for sexuality.

Very little work has been done to ascertain the location of haplonts in *N. crassa*. Three spores, the location of which was determined in each case, from each of two different asci, were germinated. The results obtained by growing these haplonts in all possible combinations and with known haplonts *A* and *B* of this species further uphold the theory that the segregation of the sex factors takes place in the second mitosis.

SUMMARY

In *Neurospora sitophila*, the ascus is long and narrow and the spindle of the first mitosis is longitudinally placed. The resulting daughter nuclei come to rest some distance apart, one above the other in the ascus. The second division spindles are also longitudinal.

The four resting nuclei are bottle-shaped and have two long horn-like appendages protruding from the neck. The spindles of the third division are transverse and are widely separated.

Spores of known position were isolated from six asci of *Neurospora sitophila* and every possible combination made in cultures. It was found that the spore haplonts may be arranged in the ascus in either one of the four following combinations, *AA*, *BB*, *AA*, *BB*; *BB*, *AA*, *BB*, *AA*; *AA*, *BB*, *BB*, *AA*; or *BB*, *AA*, *AA*, *BB*, where *A* and *B* are reciprocal as to their sexuality.

Since the spores carrying like factors for sexuality arrange themselves in pairs along the length of the ascus, the segregation of the sex factors must take place in the second mitosis. Had this factor been segregated in the first division, the four spores at one end of the ascus would be of one sex and the four spores at the other end of the opposite sex. Should this segregation take place in the third mitosis, however, the spores of opposite sex would alternate throughout the length of the ascus, which would be contrary to the experimental results.

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WASHINGTON, D. C.

EXPLANATION OF PLATE 2

Fig. *A*. Resting daughter nuclei, two-nucleate stage. Nuclei some distance apart.

Fig. *B*. Second division in metaphase stage. The spindles of this mitosis are longitudinal.

Fig. *C*. Four-nucleate stage. The second nucleus counting from above lay at a slightly higher focus in the section.

Fig. *D*. Third division, anaphase stages showing the even distribution and transverse orientation of spindles. The lower spindle is in the same ascus but in another section. The sporeplasm is divided into four regions by large vacuoles.

Fig. *E*. Delimitation of spores. The portions lying in a higher focus in the section are more heavily shaded. The nuclei are much elongated and end in a curved beak.

Fig. *F*. Later stage in delimitation of spores showing retraction of nuclei. The hooked end of such nuclei still visible at the apex of each spore.

Fig. *G*. Karyokinesis in spores.

Fig. *H*. Mature spores, showing two nuclei.

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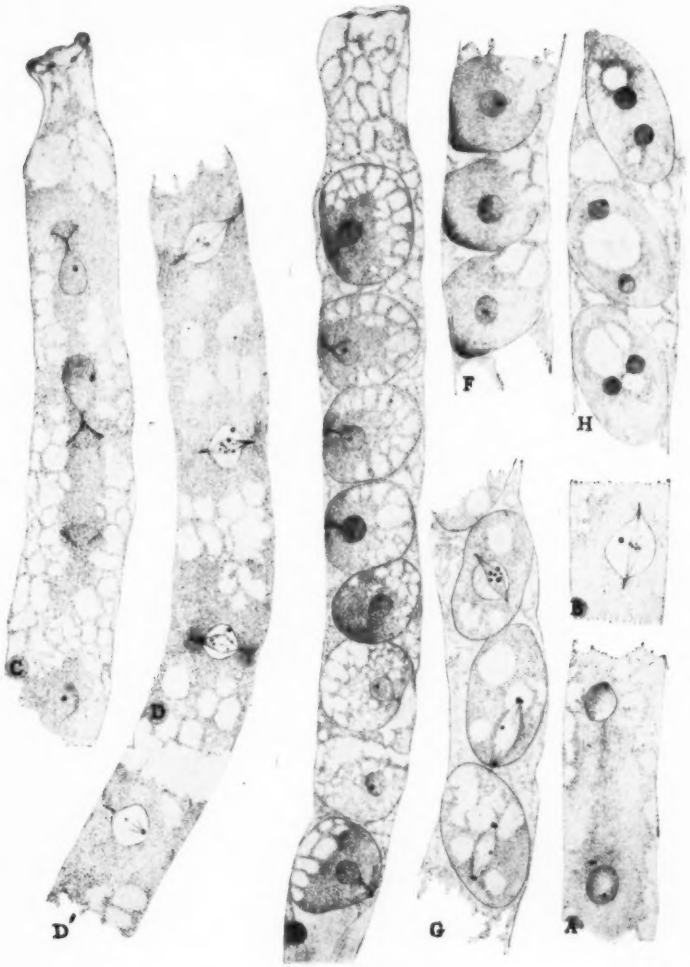

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SPORE FORMATION IN ASCI WITH FEWER THAN EIGHT SPORES

B. O. Dodge

The fusion nucleus in the ascus of the higher ascomycetes regularly divides three times in succession. In case more than eight spores are to be developed in the ascus, the eight nuclei undergo further simultaneous divisions so that 16, 32, or more nuclei are formed before spore delimitation. The writer has counted 1024 spores in certain asci in *Thelebolus stercoreus*. There must have been at least 1024 nuclei resulting from the seven additional divisions in the ascus before spore formation. Spore formation in such asci has been worked out by Overton (**6**), Sax (**7**), and others. In case an ascus should develop fewer than eight spores, as in *Phyllactinia corylea*, for example, which very commonly develops two spores, Harper (**4**) has shown that six of the nuclei degenerate so that each spore contains only one nucleus at its origin. Komarnitzky (**5**) further confirmed this doctrine of degeneration of nuclei in connection with *Verpa bohemica* (*Morchella bispora*) the ascus of which has only two spores. Komarnitzky's figures are not wholly conclusive; nevertheless, it would seem from his discussion that six of the nuclei degenerate, only two taking part in spore formation, one for each spore. Faull (**3**) has studied two species of *Laboulbenia*. Most species of this order develop only four spores in an ascus. The four spindles of the third division are longitudinal. The nucleus formed at the upper end of each spindle migrates to the top of the ascus and degenerates. One of the four nuclei from the lower ends of the spindles then is included in each spore as it is cut out.

Wolf was the first to report the inclusion of two nuclei regularly at the formation of each spore in an ascus. The ascus of *Podospora anserina* usually forms only four spores. Wolf (**9**) says that two of the eight nuclei in the ascus enter each spore. Inas-

much as he failed to furnish all of the details of nuclear behavior leading up to spore formation, and as this process was contrary to what had heretofore been reported in other species, his paper has not perhaps received the attention which it deserves. Two or three other cases where two or more nuclei take part in spore formation have been mentioned incidentally merely as abnormalities. The writer (**2**) has recently studied spore formation in the four-spored ascus of *Neurospora tetrasperma* Shear and Dodge (**8**). It has been found that the eight nuclei of the ascus come together in pairs, side by side, so that two nuclei, one of each sex, take part in the formation of each spore. Occasionally an ascus may develop five spores. In such case two of the spores will each contain only one nucleus. Whereas *Neurospora sitophila* is heterothallic, mycelia derived from the binucleate spores of *N. tetrasperma* are homothallic. On the other hand, a mycelium from a uninucleate spore of this species does not develop perithecia when grown alone. When two such mycelia of opposite sex are grown together on a suitable medium, ascocarps with four-spored asci are produced. Should an ascus develop only two large spores, each one will contain four of the eight original nuclei. A mycelium from such spores is totipotent.

A number of years ago while working on leaf forms of *Gymnosporangium* on *Chamaecyparis* in the pine barrens of New Jersey, the writer found a tree at Lakehurst which was badly infected with what appeared to be a new species of *Keithia*. A preliminary study of nuclear behavior in the ascus convinced the writer at the time that each of the two spores in the ascus originally contains four nuclei at the time the spore is cut out. As the fixation of this material was not wholly satisfactory for cytological study so that the details of nuclear behavior at various stages leading up to spore formation could not be determined readily, this material was turned over to Dr. J. F. Adams (**1**), who continued the study on additional material and later published a short account of the parasitism and general morphology of the fungus and described it as a new species, *Keithia Chamaecyparissi*.

The writer has recently reëxamined his old preparations as well as the slides kindly loaned him by Dr. Adams. It would appear

that in this species of *Keithia* each spore contains originally four nuclei. The four nuclei coöperating in spore formation seem to be arranged more or less equatorially in the young spore, the beaks pointing outward. Later the nuclei become distributed irregularly and finally crowd toward the upper or tip end of the spore. Whether or not the four nuclei undergo further division just before the septum is laid down, cutting off a very small segment at the upper end of the spore, cannot be determined from this material. In any event, the small segment contains at least two nuclei. Additional material has been recently obtained from Lakehurst and it is hoped that further information will soon be available. The point to be emphasized is that here we have another species of ascomycete with fewer than eight spores and in the delimitation of the ascospores of which more than one nucleus is regularly concerned.

It is well known that where a species commonly develops eight spores in an ascus, occasionally one or more of the spores for some reason will fail to reach full maturity, or abort after it has been delimited. The powdery mildews which develop fewer than eight spores are in still another category since certain nuclei degenerate without first cutting out spores. *Keithia thujina* also develops only two spores, and there are several other well-known species of ascomycetes which usually mature fewer than eight spores in an ascus. The question arises, is the failure in a particular case to mature eight spores due (1) to an abortion of certain spores after their delimitation, (2) do certain of the nuclei degenerate, or (3) do two or more nuclei coöperate in the processes of spore delimitation? In *Neurospora tetrasperma* the inclusion of two nuclei in each spore serves a very useful purpose since the factors for sex, as it may be called for convenience, are segregated out before the spores are delimited. The distribution of the nuclei in the ascus and the orientation of the spindles is such that two nuclei of opposite "sex" can readily coöperate in cutting out a spore. On rare occasions *Keithia* develops more than two spores. Sections of an ascocarp show one ascus in which four spores were originally delimited. The largest spore contains three nuclei, two spores have two nuclei each, and the smallest spore only one. Perhaps such small spores lacking the full com-

plement of nuclei possess a different genetic constitution. May they not sometimes even be heterothallic?

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NOTES ON THE PARASITOLOGY OF SCLEROTIUM ROLFSII

RUTH DAVIS PAINTIN

(WITH PLATES 3 AND 4 AND 1 TEXT FIGURE)

The many studies of *Sclerotium Rolfsii* Sacc. which have been made since it was first reported by Rolfs (**3**) in 1893 seem to indicate that the fungus is a weak parasite with no host preference. It has previously been recorded as parasitic on about one hundred and forty species and varieties of flowering plants, including such important crops as: beans, potatoes, cowpeas, peppers, melons, and tomatoes. It is the cause of considerable annual loss in corn, wheat, cotton, rice, and tobacco. *S. Rolfsii* also infects numerous ornamental plants, for example: *Campanula*, *Coreopsis*, *Chrysanthemum*, *Delphinium*, *Daphne*, *Dahlia*, carnation, sweetpea, and violet.

In general the losses from this disease are not severe, except in isolated fields. However, the percentage of damage seems to be gradually increasing according to the reports of the Plant Disease Survey, and its control is becoming a serious agricultural problem especially in the southeastern United States.

Although *S. Rolfsii* is especially prevalent in the southeastern United States, recent reports show isolated occurrences in Pennsylvania, Ohio, Illinois, Indiana, Nebraska, and California. It has also been reported from China, Australia, and numerous islands of the Pacific and Atlantic oceans. It has been found in Uganda, Africa, and in the Vorstenlanden. The writer isolated the fungus from a diseased potato purchased in Chicago, but shipped from Virginia.

NEW HOSTS

A series of soil inoculation experiments was conducted in the green house using sclerotia from cultures isolated from Lima bean in Florida by G. F. Weber and from Potato in Texas by J. J. Taubenhaus.

From these experiments the following named plants, not previously reported, have been determined by the writer as hosts:

Begonia sp.
Brassica Rapa L.
Brassica sp. (var. Improved Ostrich Plume)
Bryophyllum pinnatum Kurz.
Chenopodium sp.
Cichorium Endivia L.
Coleus Blumei Benth.
Commelina sp.
Cucurbita Pepo Baily (var. *ovifera*)
Dolichos multiflorus Torr. & Gray (var. Early Speckle)
Echinochloa frumentacea Link
Hordeum sativum Jess. (beardless)
Impatiens Sultani Hook.
Melilotus alba Desr.
Myriophyllum sp.
Ornithogalum umbellatum L.
Oxalis sp.
Petroselinum hortense Hoffm.
Phaseolus multiflorus Willd.
Pteris longifolia L.
Reseda odorata L.
Ricinus communis L.
Salvia officinalis L.
Thymus vulgaris L.
Triticum aestivum L. (var. Marquis)
Tropaeolum sp.
Vinga sinensis Endl. (var. Clay)
Zea everta Sturtev.

METHODS

Very little histological work has been reported in connection with this fungus. The only record of a stain used is contained in a brief note by Rolfs (**3**) who mentions picrocarmine.[1]

For the purpose of histological study host tissues were prepared as follows: The materials were killed in Flemming's solutions, weak or strong, or in chromacetic acid. The time allowed for the action of these various agents was about 24 hours. When Flemming's stronger solution was employed in preparing the material, mixture *A*, containing osmic acid, was used in combination with solution *B*, for one hour. For the remainder of the time the material was kept in solution *B* which lacked osmic acid. This practice was employed to prevent darkening of tissues. If bleaching was necessary, a mixture of hydrogen peroxide and 50 per cent alcohol solution was used. Paraffin sections were made 10 to 12 μ in thickness. Dickson's (**1**) differential stain, with some modifications, was found most satisfactory. The procedure was: magdala red, ten minutes; rapid rinse in 85 per cent alcohol; light green, one half to one minute; carbol turpentine or 100 per cent alcohol for clearing; mount in balsam. This process is differential, usually staining the fungus a reddish purple, while the parenchyma takes on a light

[1] B. B. Higgins' paper, Physiology and parasitism of *Sclerotium Rolfsii* Sacc. Phytopathology **17**: 417–448. 1927, appeared after this paper was in proof.

green color. The xylem strands stain reddish, thereby not making a clear color contrast between the fungal hyphae and the lignified tissue.

PATHOLOGICAL HISTOLOGY OF THE HOST

Abrasion or wounding of the host is not necessary for infection. Penetration is accomplished either directly by piercing or by a dissolution of the cell wall. The fungus usually gains entrance into the plant by mass action (PLATE 3, FIG. 2; TEXT FIG. 1) a little below the surface of the soil. However, an exception to this was noted in the case of a single hypha which was observed to have entered through a hair cell into the cortex of the stem of cowpea. Also single, intercellular hyphae, which dissolved the middle lamellae, were observed to have passed through the walls

FIG. 1. Cross-section of the stem of cowpea showing the typical radial fans or mats of mycelium invading the cortex. × 700. Camera lucida. *a*, hyphae constricted between cells.

into the cells. In general, however, the hyphae enter the epidermal cells in a mass, grow abundantly through the cortex both intercellularly and intracellularly and cause a very severe infection (PLATE 4). This observation differs from the statement of Edson and Shapovalov (2) that hyphae usually do not enter the tissue directly, but a disorganization takes place due to digestive enzymes.

The mycelium was observed in the stele of the crown of cowpea and velvet bean seedlings, where the hyphae travel up into the stems (PLATE 3, FIGS. 3, 4) and down into the roots of the plants. Frequently the fungus brings about a complete disintegration of the central cylinder. In the region of initial infection the cortex is usually most severely invaded. The hyphae either travel through the tissue from cell to cell, piercing (PLATE 4, FIG. 1*a*) or dissolving (PLATE 4, FIG. 2*a*) the cell wall, or they grow in the cell wall, dissolving the middle lamella (PLATE 3, FIG. 1) and often distending the walls with the mass of crowded hyphae. More often the hyphae pass through the cell wall without constriction (PLATE 4, FIG. 1*b*), as has been observed by Small (**4**), but they may be constricted (TEXT FIG. 1*a*). Hyphae in large fascicles may travel up and down a stem of cowpea, invading in a mass. The growing hyphae exert a considerable pressure as is shown by the frequent distending or bulging of the host cell walls when the tips of hyphae come in contact with them. In the final phase of severe infection all that remains of the host is a few disorganized fragments of the cell walls among the extensive mat of hyphae.

The writer wishes to express appreciation for advice and supervision of this study by Professor Alfred Povah; also to acknowledge the courtesy of Messrs. Taubenhaus, Whetzel, Weber, Edgerton and Moreland for sending cultures.

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EXPLANATION OF PLATES

PLATE 3

Fig. 1. Intercellular mycelium in the stem of cowpea showing vacuoles and nuclei or food bodies. × 1500.

Fig. 2. A longi-section through a velvet bean stem, near the region of infection, showing the entrance of the fungus in a mass. × 1300. Projection microscope.

Fig. 3. A longi-section of the stele of an infected cowpea stem. × 1000. Camera lucida.

Fig. 4. A longi-section of the stele of an infected velvet bean stem. × 1300. Camera lucida.

PLATE 4

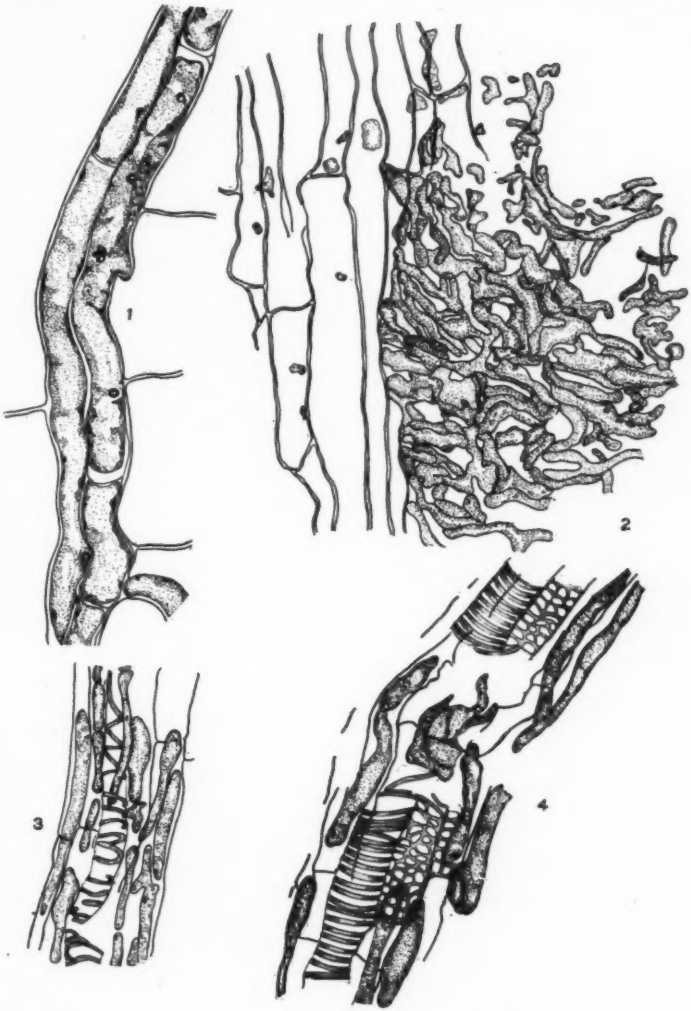
Fig. 1. Cross-section of the cortex of an infected cowpea stem, near the crown. × 1300. Projection microscope.

a, wall pierced by hyphae.

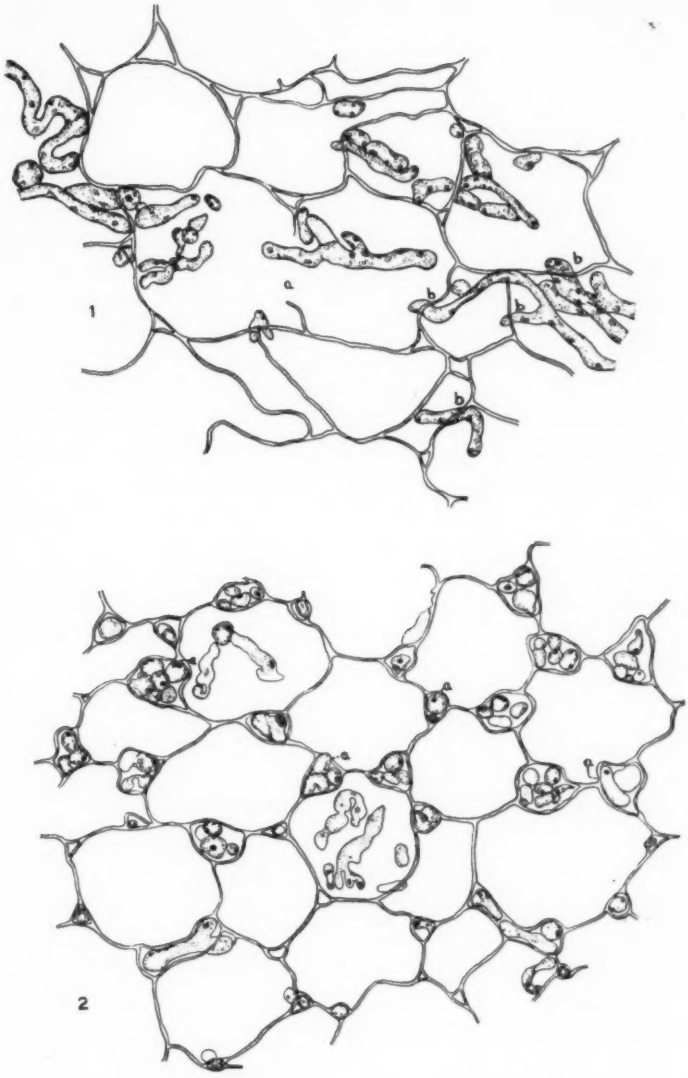
b, hyphae passing from cell to cell without constriction.

Fig. 2. Cross-section of the cortical region of a heavily infected velvet bean stem, showing the intercellular mycelium. × 1300. Projection microscope. *a*, walls dissolved by hyphae.

(Drawings reduced one-third in reproduction.)



STEM SECTIONS OF VELVET BEAN AND COWPEA INFECTED WITH SCLEROTIUM ROLFSII



CROSS-SECTION OF INFECTED STEMS OF COWPEA AND VELVET BEAN

MYXOMYCETES FROM BRITISH GUIANA AND SURINAM

FRANK A. GILBERT

During the winter of 1923–24, Dr. David H. Linder, while a Sheldon Traveling Fellow from Harvard University, made a collection of fungi from British Guiana and Surinam. In this collection were a number of Myxomycetes which were turned over to the writer for identification and are now in the Farlow Herbarium. These Myxomycetes are not new, nor even extremely rare, in fact the majority are widely distributed throughout the world, but coming as they do from that part of South America where few collections of fungi have been made, their occurrence is of geographical importance and considerably extends the ranges of a number of species.

In this list, which follows, the locality, date, and collector's number are given under each species. Unfortunately, notes upon the character of the substratum were not taken in most cases.

CERATIOMYXA FRUTICULOSA (Muell.) Macbr.
Bartica, B. G.; December 1923; Linder *608*.

CERATIOMYXA FRUTICULOSA (Muell.) Macbr. var. FLEXUOSA Lister.
Bartica, B. G.; December 1923; Linder *608a*.
Tumatumari, B. G.; September 15, 1923; Linder *216*.

PHYSARUM BOGORIENSE Racib.
Botanic garden, Paramaribo, Surinam; November 8, 1923; Linder *368*.
Plantation Vryheid, B. G.; February 1, 1924; Linder *878*.

PHYSARUM POLYCEPHALUM Schw.
Plantation Vryheid, B. G.; February 1, 1924; Linder *919*.

PHYSARUM TENERUM Rex.
Bartica, B. G.; January 20, 1924; Linder *733*.

TRICHAMPHORA PEZIZOIDEA Jungh.
Botanic Garden, Surinam; November 1923; Linder *321.*

PHYSARELLA OBLONGA (Berk. & Curt.) Morg.
Plantation Vryheid, B. G.; February 28, 1924; Linder *1001.*

DIDYMIUM NIGRIPES (Link) Fr.
Bartica, B. G.; December 19, 1923; Linder *491.*

COMATRICHA TYPHOIDES (Bull.) Rost.
Plantation Vryheid, B. G.; February 16, 1924; Linder *962.*

TUBIFERA STIPITATA (Berk. & Rav.) Macbr.
In wet forest, near botanic garden, Surinam; November 4, 1923; Linder *317.*

LYCOGALA EPIDENDRUM (Buxb.) Fr. var. EXIGUUM Lister.
Bartica, B. G.; January 19, 1924; Linder *784.*

ARCYRIA CINEREA (Bull.) Pers.
Botanic garden, Paramaribo, Surinam; November 7, 1923; Linder *356.*

ARCYRIA CINEREA (Bull.) Pers. var. DIGITATA G. Lister.
Decayed log, Tumatumari, B. G.; September 15, 1923; Linder *217.*

ARCYRIA DENUDATA (L.) Wett.
Bartica, B. G.; January 17, 1924; Linder *739.*
Botanic garden, Paramaribo, Surinam; November 1, 1923; Linder *319.*

PERICHAENA CHRYSOSPERMA Lister.
Plantation Vryheid, B. G.; February 28, 1924; Linder *995.*

Obviously a list of this sort can not enumerate the myxomycetous flora of the region, for in a general collection such as that made by Dr. Linder, it is impossible to gather a large number of species of every order; and the Myxomycetes, because of their inconspicuousness and the difficulties incidental to their gathering and preservation in the field, are collected even less than the other groups. As a result, tropical specimens are poorly represented in most herbaria and each collection adds a little more to our knowledge of their geographic distribution. For that reason this list, even though small, is of some general interest.

LABORATORIES OF CRYPTOGAMIC BOTANY,
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A NEW GENUS OF THE SUBFAMILY NITSCHKIEAE

R. CIFERRI

(WITH 1 TEXT FIGURE)

The specimen on which the present contribution is based is a part of the Reliquiae Massanae preserved in the Mycological Herbarium of the School of Viticulture and Enology in Alba (Italy), and lacks reference to host and locality, and time of collection.

The fungus was found on dry decorticated branches. The perithecia are coriaceous-carbonaceous, black, cupulate, not distinctly ostiolate, composed of a nearly hemispherical superior ascigerous cavity and of a sterile basal part, of plectenchymatous tissue. The perithecia and hyphae are furnished with black, sharp-pointed and minutely tuberculated prickles and have a very light to nearly brilliant black metallic iridescence. The ascus contains eight ellipsoidal or ovoidal, inordinate, brownish or blackish, smooth, continuous spores.

Following the complete monograph on the subfamily Nitschkieae by Fitzpatrick (**1**), the fungus in its possession of perithecial prickles is near to the genus *Acanthonitschkea* Spegazzini (**2**). It differs from it in spore characters and bears the same relation to *Tympanopsis* Starbäck (**3**) that *Acanthonitschkea* bears to *Nitschkia*. Since no genus in the group will include the species, it seems desirable to found for it a new genus and this is dedicated to the monographer of the subfamily, H. M. Fitzpatrick.

Fitzpatrickia n. gen. (clar. mycol. H. M. Fitzpatrick dic.)

Astromaticus; peritheciis superficialibus hemisphaericis, aggregatis, leviter tuberculatis, indistincte ostiolatis, coriaceo-carbonaceis, nigris, spinulatis; hyphis subiculi iridescente-nigrescentibus, ramificatis, septatis, leviter tuberculatis, parce spinulatis; ascis clavatis, aparaphysatis, octosporis; ascosporis irregulariter dispositis, brunneolis vel fuscis, continuis, levibus; spinis rigidulis, erectis vel suberectis, continuis, non ramificatis, acutis, nigro-iridescentibus.

Fitzpatrickia Massae n. sp. (memoria Doct. Caroli Massae dic.)

Peritheciis aggregatis, rotundato-cupulatis, nigris, iridescentibus, leviter tuberculatis, indistincte ostiolatis, 320–460 μ diam., spinulatis; ascis clavatis vel elongato-clavatis, 19.5–27 $\times$ 5–8.5 μ, aparaphysatis, octosporis, mox evanescentibus; ascosporis ellipsoideis vel ovoideis, saepe inequilateralis, non seriatis, continuis,

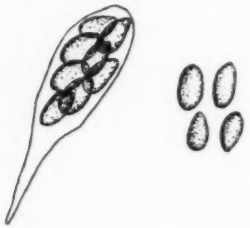

FIG. 1. Ascus with spores FIG. 2. Ascospores

flavo-brunneis usque ad fuscidulis, 7.5–9 $\times$ 2.5–3.5 μ, levibus; hyphis brunneis vel nigrescentibus, armatis; spinis elongatis, rigidulis, continuis, non ramificatis, leviter tuberculatis, 140–190 $\times$ 8–15 μ.

Hab. in ligno decorticato, leg. C. Massa in loco indeterminato.

The specimen is preserved in the Mycological Herbarium at Alba, Italy.

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THE FRUITING OF COLLYBIA DRYOPHILA IN PURE CULTURE[1]

R. F. Poole

(With Plates 5 and 6)

Collybia dryophila was obtained in pure culture by planting on prune agar, mycelium and rhizomorphs from dewberry roots, where it is parasitic. The mycelium grew abundantly on a variety of acidified culture media, including sweet potato, apple, rice, and barnyard manure, but mushrooms were not obtained on any of the above media. They developed slowly on cooked, dead, dewberry roots, but became very abundant after prune medium was added. The plants were very uniform in such characters as color, composition and arrangement of parts, and varied only in physical sizes.

The definite uniformity of the plants was so striking as to indicate that similar studies of related strains on a standard medium may lead to a definite understanding and explanation of the confusion and uncertain definition that exists in such groups as *Collybia dryophila* and *Armillaria mellea* at the present time. It is hoped that the results presented in this paper on the fruiting of *Collybia dryophila* in pure culture may be of some assistance to those interested in studying other related strains and groups in pure culture.

The culture medium used in growing the mushrooms was prepared by cutting, into small sections, parts of dead roots and canes of the dewberry. To this was added 200 cc. of prune medium, which was prepared by cooking 150 grams of large prunes in a liter of water. The combined prune dewberry medium in two-liter flasks was sterilized for one hour at the ordinary temperature and pressure. Cultures were started in July, August, and October. They were maintained at 25 degrees centigrade in candle light. There was simultaneous and abun-

[1] Published with the approval of the Director of the North Carolina Experiment Station as paper number 21 of the Journal Series.

dant growth of both mycelium and rhizomorphs. They became very prominent after fourteen days, when a large amount of spawn was transferred to the flasks.

The first production of plants was obtained in 30 days, but maximum production was obtained after a growth of 60 to 90 days. In a two-liter flask, 17 well-developed mushrooms were grown in two months. In six flasks 58 mushrooms were removed in the same period. The cultures have continued to produce new rhizomorphs and mushrooms for six months.

Although the mature caps varied from 2 to 8 cm. in diameter, the extremes were rare, and the average was between 4 and 5 cm. Sometimes when 8 to 12 plants were developed together in the same flask some were more vigorous than others (PLATE 5, FIG. 1). The color changes were always uniform. The same facts were noted in the form, size and arrangement of the gills, flesh and heterogenous tissues. The sizes of caps and stems were the notable differences.

TABLE I

MEASUREMENTS OF ELEVEN PLANTS GROWN FROM DECEMBER 23 TO 27 IN A TWO-LITER FLASK

Plant	Pileus (diameter) cm.	Stype (length) cm.	Stype (diameter at base) mm.	Stype (diameter at cap) mm.
1	8.0	10	20	12
2	4.5	11	10	7
3	4.5	10	15	6
4	6.0	10	12	11
5	4.5	10	12	7
6	4.5	10.5	11	7
7	4.0	9	12	8
8	3.2	9.5	7	5
9	3.0	9	9	5
10	2.5	7	9	4
11	3.0	7	8	2

The measurements of eleven plants grown in single flasks from December 23 to 27 are given in table I. In this yield of eleven plants the caps varied from 2.5 to 8 cm. in diameter but five of this number measured only from 4.0 to 4.5 cm. The eleven stems measured from 7 to 11 cm. in length, while seven varied from only 9 to 10 cm. The bulb-like base varied from 8 to 20 mm. in diameter. The even stem near the cap varied from 2 to 12 mm., but seven stems measured from 4 to 7 mm.

The rate of growth of the mushrooms was readily followed in the flasks, where the first signs were small fuzzy-like tumors on the rhizomorphs. These were mostly numerous, but only a few got any larger than the button, where they wilted and dried. The matured plants grew slowly in the beginning and for the first 48 hours. Later growth was quite rapid in comparison to the first two days. The stems developed thickness at first and during this period of development the tomentose covering was prominent on the young stems. This became less distinct as the stem extended in length, but the striations which were faint the first two days became very pronounced during the next 48 hours. The stem of the young plant was mostly white and began to tan and brown from the base upward as the plant matured. It sometimes became equal in color to the cap. The smaller size of the stem at maturity was due to the rapid development of the stem into length only a few hours previous to maturity.

TABLE II

THE RATE OF DEVELOPMENT OF THREE *Collybia dryophila* MUSHROOMS IN PURE CULTURE

Plant no.	Date	Time	Pileus (diameter) mm.	Stype (length) mm.	Stype (diameter at base) mm.	Stype (diameter at cap) mm.
1	Jan. 12	5 P.M.	Small tumors			
	" 13	10 A.M.	Button	2	1	.5
	" 14	10 A.M.	5	11	4	3
	" 14	5 P.M.	7	15	4	3
	" 15	10 A.M.	10	27	4	2
	" 16	10 A.M.	16	50	3	2
2	Jan. 12	5 P.M.	Small tumors			
	" 13	10 A.M.	Button	2	1	.5
	" 14	10 A.M.	9	20	5	4
	" 14	5 P.M.	10	22	6	5
	" 15	10 A.M.	28	57	6	4
	" 16	10 A.M.	44	90	6	3
5	Jan. 12	5 P.M.	Small tumors			
	" 13	10 A.M.	Button	2	1	.5
	" 14	10 P.M.	12	25	10	5
	" 14	5 P.M.	15	26	12	5
	" 15	10 A.M.	30	65	12	7
	" 16	10 A.M.	54	100	12	6

The growth of the cap can be described as gradual and uniform from the beginning. The form, somewhat gibbous, was main-

tained throughout. The cap was whitish for one day, then it became light tan and shades of brown in 24 hours, and afterwards changed only slightly to a deeper brown in the center. The greatest growth of the cap was during the last 48 hours before maturity which was always between 4 to 5 days.

The development of the mushroom is best observed in plate 5, figure 1, where the new tumor-like button and one- and two-day-old plants, photographed 10 A.M., December 24, are shown. Successive figures 2 and 3 show the later developments of the same plants at 10 A.M. on December 25 and 26, respectively.

Some measurements on different days of plants in another flask are given in table II. The most interesting part of these data is the rapid growth of the plants during the second 48 hours, between January 14 and 16. Although the complete development of the plant was attained in 4 to 5 days, best sporing occurred when the plants were not disturbed for 48 hours longer. The plants began to slowly dry out after they were fully matured.

Sometimes there was an unequal development of subcespitose plants on the same rhizomorph (PLATE 5, FIG. 1). The two plants on the extreme left were the same one and two described in table II. In the beginning these plants were of equal size throughout, but at maturity the stem of plant 1 was 40 mm. shorter and the cap 28 mm. less in diameter than plant 2. This is not surprising since there were 21 buttons in this flask January 12, and only 8 matured, counting plant 1, which was below normal size. Uniformity of growth was not always interfered with when two plants were developed side by side from the same rhizomorph as is shown in figure 2. The greatest uniformity of size, as well as other characters, was obtained when solitary plants were grown (Fig. 3).

DESCRIPTION OF PLATES

PLATE 5

Fig. 1. Buttons and small mushroom growing on old dewberry roots saturated with prune medium in two-liter flasks. Photographed 10 A.M., December 24.

Fig. 2. The same mushrooms photographed 10 A.M., December 25.

Fig. 3. The same mushrooms photographed 10 A.M., December 26.

1 2 3

COLLYBIA DRYOPHILA

1 2 3

COLLYBIA DRYOPHILA

PLATE 6

Fig. 1. Mushrooms of different sizes on same rhizomorphs. Subcespitose plants on extreme left show big variation in size at maturity although of same size in the beginning.

Fig. 2. Mushrooms, however, may attain equal size without one interfering with the other in growth.

Fig. 3. A solitary mushroom.

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THE UREDINIA OF CRONARTIUM COMANDRAE AND MELAMPSORA MEDUSAE

E. H. Moss

Among the rusts (Uredinales) the family Melampsoraceae is of great interest because of the variety of host relationships and the diversity of forms exhibited by its various members. Attempts have been made to give expression to natural affinities among genera of the family, but, unfortunately, many of these attempts have been based upon superficial observations or upon incorrect interpretations of certain morphological characters. It is apparent, therefore, that a more thorough investigation of these rusts should precede further endeavors to express natural relationships in a phylogenetic classification or otherwise.

In a recent paper (**4**), attention was directed to the uredinial stage of one subfamily of the Melampsoraceae, namely, the Pucciniastreae, and it was concluded (contrary to certain earlier reports) that the uredinia of representative forms of this subfamily are strikingly similar in their mode of development. The present communication deals with a member of each of two other subfamilies of the Melampsoraceae, namely, the Cronartieae and the Melampsoreae, and the main conclusion reached is that the uredinia of these species develop in essentially the same manner as in the Pucciniastreae. In connection with the following descriptions of *Cronartium Comandrae* and *Melampsora Medusae*, it has been considered unnecessary to provide illustrations, because very similar stages in development are fully figured in an earlier paper (**4**).

Cronartium Comandrae Peck

This species was obtained on *Comandra Richardsiana* Fernald in the jack-pine forest of central Alberta. Uredinia were collected on July 22, 1926, fixed in medium chromacetic acid, and subsequently embedded in paraffin. Microtome sections were stained with safranin and light green, a combination that

proved very satisfactory for the interpretation of young uredinia.

The uredinium develops from a hyphal plexus beneath a stoma of the host leaf. Vertical hyphae arise in palisade fashion under the epidermis and become divided each into three enlarged cells, namely, peridial, intercalary and sporogenous cells. It is probable that peridial and intercalary cells are sister cells, but convincing evidence of this has not been found in my preparations. At this early stage, the uredinium closely resembles that of *Uredinopsis*, as figured in an earlier paper (**4**). The peridial cells have small nuclei and large vacuoles; the intercalary cells have degenerate nuclei and cytoplasm that stains faintly; and the sporogenous cells are characterized by large nuclei and deeply staining cytoplasm. At a slightly later stage in development, the intercalary cells collapse, due to the outward growth of the basal sporogenous cells. Thus the intercalary cells function as space-formers and also as "disjunctive" cells (Kursanov's term), serving to separate the peridial from the sporogenous cells. A conjugate nuclear division then takes place, one pair of daughter nuclei passing into the "bud," after which a septum is formed between the "bud" and the mother cell. The bulbous cell which thus arises from the basal cell is a spore-initial; following further expansion and conjugate nuclear division, it becomes divided by a transverse wall into stalk cell and young spore. Before the spore thus formed has reached maturity, a second spore-initial may arise by a "budding" process from the basal cell, and still later additional spores may develop from the same sporogenous cell. As a consequence of the pressure exerted by the developing spores, the peridial cells become somewhat flattened and the epidermis bulges outwards, and finally the epidermal and peridial cells that cover the central part of the sorus are ruptured, thus allowing the spores to emerge.

It should be pointed out that the foregoing description differs from that given by Colley (**1**) for *Cronartium ribicola* Fischer in one important respect. Colley does not recognize in the uredinium a layer of disjunctive cells, although his illustration of a young sorus shows three-celled columns with subterminal cells that closely resemble the disjunctive cells as I have observed them in *Cronartium Comandrae*. The subterminal cells are

interpreted by Colley as the first spore-initials, and no mention is made of disjunctive cells in connection with the separation of the peridium from the underlying spore-initials and spores.

MELAMPSORA MEDUSAE THÜM.

A collection of young uredinia of this species was made in the Timagami Forest Reserve, Ontario, on August 29, 1925. Some of the material was carefully fixed in the field and later used in making preparations suitable for the study of the sori.

The early stages in development of the uredinium are essentially the same as described above for *Cronartium*. The peridial and intercalary cells of the three-celled columns are considerably smaller and less conspicuous than in *Cronartium* and have a very transitory existence. These cells are, however, quite as easily observable in the young sorus as are the corresponding cells in the uredinium of *Hyalopsora* (**4**). Spore-initials arise from the basal cells by the characteristic "budding" process, and the pressure exerted by the growth of the first spore-initials effects the early collapse of the intercalary cells. Unlike *Cronartium Comandrae* and the Pucciniastreae, *Melampsora Medusae* produces paraphyses in its uredinium. These organs seem to arise, in the first instance, from the border of the rather young uredinium after a number of spores have been formed in the central part. The paraphyses arch over the developing spores, forming a fringe round the mouth of the newly opened sorus. During the development of the peripheral paraphyses the peridial cells become more or less crushed, and for this reason these cells can best be observed in the very young uredinium. Later, paraphyses commonly develop in the central part of the sorus as well as towards the border. The paraphyses seem to arise from basal cells, either peripheral ones which are not sporogenous, or more central ones which have already produced one or more spore-initials. Whether the paraphysis is separated from the basal cell by a septum is difficult to ascertain owing to the constricted nature of the lower end of the paraphysis, but such a septum does not appear to exist.

The uredinium of *Melampsora* has been generally considered to be without a peridium. However, as long ago as 1899,

Klebahn (**2**) observed that certain species of *Melampsora*, namely, *M. populina* and *M. Lini*, have evanescent peridia. Klebahn was inclined to think that a peridium is absent in the uredinium of *M. Laricis-capraearum* of the willow. More recently Kursanov (**3**) has described a uredinial peridium for *Melampsora Helioscopiae* Winter on *Euphorbia* and has concluded, from an examination of mature uredinia of *Melampsora* on *Salix* and *Populus*, that peridia are also present in these species. There is abundant evidence therefore that a peridium characterizes the uredinium of *Melampsora*, but whether disjunctive cells occur below the peridium is a matter requiring further investigation. According to my observations, disjunctive cells are formed in *M. Medusae*, but according to Kursanov no such cells separate the peridium from the sporogenous cells in *M. Helioscopiae*.

DISCUSSION

Representatives of the Pucciniastreae (**4**), Cronartieae and Melampsoreae that have been examined by the writer are essentially alike in the mode of development of their uredinia, having in common the following features:

(1) A peridium is present and consists of the terminal cells of three-celled columns which arise in the young sorus.

(2) The basal cells of the columns give rise to spore-initials by upward growth or "budding."

(3) The intercalary cells of the columns disorganize and serve to separate the peridium from the basal cells and to make space for the initial growth of the first spore-initials.

It is true that marked differences exist among the uredinia of these rusts, for example, differences in size and shape of the mature pustule, in spore characters, in degree of persistence and differentiation of the peridium. These characters are undoubtedly of the greatest diagnostic value in taxonomy, but from a phylogenetic point of view they are probably of secondary importance in comparison with the fundamental features that have been emphasized in this paper. Even the paraphyses of *Melampsora*, particularly if these are to be regarded as the homologues of spore-initials or of spores, may be of less importance in connection with natural relationships than is the evanescent peridium with its associated disjunctive cells.

The bearing of the conclusions of this paper upon the question of affinities among the rusts, and upon the problem of the relative primitiveness of various types of sori will be determined only as a result of further investigation in the Melampsoraceae, including the subfamily Chrysomyxeae, as well as in other groups of the Uredinales.

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NOTES ON ARCTIC UREDINALES[1]

J. C. Arthur

The plants brought back from northwestern Greenland in the Danish Jubilee Expedition of 1920–23 have been critically searched for micromycetes by Mr. J. Lind, resulting in the detection of 80 species, 5 of them being Uredinales. Mr. Lind has made his report in No. 2 of the account of the expedition (1926) and takes occasion to revise some of the taxonomy involved. As to the rusts it is only required to state here that the writer agrees entirely with the conclusions, except in the case of *Puccinia Thlaspeos*, which he believes to be quite distinct from *P. Holboellii*. The characters of the two species are given in volume 7, page 532, of the North American Flora, a publication, by the way, that is not mentioned in the literature cited.

In a later work, "The Geographical Distribution of Some Arctic Micromycetes," 1927, Mr. Lind has made a highly important contribution to the subject of plant dissemination in high northern latitudes. He points out that the strong wind of the region may carry seeds, fragments of stems and leaves for long distances over the surface of snow and ice, often depositing them in crevices or valleys where conditions are favorable for growth upon return of warm weather. For nine months of the year ice bridges the waters between islands and even the continents, so that the winds may sweep parts of flowering plants with the endophytes living upon them over a smooth dry surface in a circumpolar migration, even over such long stretches as between Greenland, Norway, Nova Zembla and Spitzbergen. This deduction is supported by citations from the writings of a number of arctic travelers. In all probability the agency of the wind is the chief, if not the only, agency for the dispersal of plants and their parasites in arctic regions, a conclusion which seems logical.

It is also pointed out that the most common arctic rusts are short-cycle species, like *Puccinia Arenariae*, *P. Holboellii*, *P.*

[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station.

Drabae (*P. Cochleariae*), *P. Cruciferarum* (*P. Cardamines-bellidifoliae*), which are readily distributed in connection with their hosts. Nine such species are known from near the arctic circle in North America.

The author's statement regarding heteroecious species, however, requires emendation. He says, "If heteroecious species of rusts were found in arctic regions, or other species of fungi the nature of which demanded that spores from one host-plant should be transferred to another in order to thrive, it would be a proof against my theory that the endophytes are spread together with their hosts in arctic countries." This statement carries the inference that heteroecious rusts require two unlike hosts in order to thrive, which is only true for a comparatively few species, for many of them can be spread and maintained indefinitely by their urediniospores.

To account for the presence of *Melampsora arctica*, which occurs on both *Salix* and *Saxifraga*, and is one of the most abundant of arctic rusts, the author assumes that portions of infected *Saxifraga*, the mycelium being perennial, will carry the rust, and upon taking root again can spread fresh spores to the willows in the vicinity. It is equally conceivable, however, that the uredinial mycelium in the willow stems may survive the winter and spread the rust directly to other willows. That method of propagation is known to be common with *Melampsora Bigelowii* in the Rocky Mountains and elsewhere.

But neither of these methods will account for the abundance in arctic regions of the heteroecious *Puccinia Polygoni-vivipari*, which has no perennial mycelium in either alternate host. The aecia which occur on *Ligusticum* have not been found as far north as the uredinia and telia. The latter were taken on Cockburn Island, far north of the arctic circle. Probably the rust is scattered by its urediniospores, which are abundant and could easily be distributed by the wind along with the seeds and dried stems of the host.

Another rust of a still different character is the autoecious *Uromyces carnea* (*Aecidium Phacae-frigidae*) which has been collected in the aecial form near the mouth of the Mackenzie River, far north of the arctic circle. The aecia arise from a

perennial mycelium, the telia are only slightly pulverulent and are not accompanied by uredinia. In this case the distribution is doubtless by means of wind, carrying the teliospores along with fragments of the plant until lodged against other plants which can be infected when new growth begins. It is improbable that the aecia play the part in distribution that they do in the willow rust, as the branches of *Phaca* can scarcely retain their vitality and start new growth as can readily be done with *Saxifraga*.

There are nine species of long-cycle rusts known from near the arctic circle in North America, the same number as of short-cycle rusts, but with the exception of *Melampsora arctica* they are not so abundant or on so many species of hosts. All of the nine species are heteroecious except two, *Uromyces carnea* and *Trachyspora Alchemillae*. In reviewing the possibilities for distribution of these long-cycle forms there seems to be no doubt that they conform in general to the same method as the short-cycle forms and are dependent upon the wind for long distance migrations.

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AN INTERESTING FERN RUST NEW TO THE UNITED STATES

H. W. Thurston, Jr.

On May 21, 1927, several members of the Department of Botany of the Pennsylvania State College, while botanizing on a rocky hillside near Howard, Pennsylvania, found a wonderfully fine stand of the common polypody, *Polypodium vulgare* L. Literally hundreds of these ferns, in fact almost every one examined, had been attacked by a rust, which was fruiting in great abundance on the over-wintered fronds and in many instances producing very noticeable necrosis.

A rust on *Polypodium vulgare* has been described by Bell,[1] from Ontario, Canada, who noted its occurrence on "fronds of the previous summer," and named it *Uredinopsis polypodophila*. Bell also suggested the possible connection of this rust with a *Peridermium* on the 2–8-year-old leaves of *Abies balsamea* which he found in close association with the fern rust and to which he gave the name *Peridermium pycnograndis*. Faull and Watson[2] transferred the fern rust to the genus *Milesina*. Arthur[3] has accepted the connection proposed by Bell and has proposed the combination *Milesia pycnograndis* (Bell) Arth.

The station was revisited and collections made on May 30, June 24, and July 14, 1927. The first collection yielded only urediniospores which agreed well with Bell's description. In the later collections a few telia were found scattered within the epidermal cells. Measurements of these were made from fixed and stained preparations and the telia were found to be 14–21 $\times$ 36–55 μ which differs slightly from the measurements reported by Arthur in the North American Flora, namely, 16–27 $\times$ 24–45 μ.

A search of the hillside and vicinity failed to reveal the presence of *Abies*, which has never been known to occur in this region.

[1] Bot. Gaz. **77**: 25. 1924.
[2] Manuscript 1925.
[3] N. Am. Flora **7**: 685. 1925.

The question, therefore, arises in the absence of specific cultures whether *Abies* is actually the alternate host, and if so, how this rust perpetuates itself in the absence of the alternate host. On July 14, 1927, infections were observed on new fronds, of the current season. Since these fronds live over the winter, it is conceivable that late summer infections by the parasite live over winter in the form of dormant mycelium, or that the urediniospores themselves live over and do not germinate until the following spring. A somewhat similar case is found in the behavior of *Hyalopsora Polypodii* (DC.) Magn. on *Filix Fragilis*.

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NOTES AND BRIEF ARTICLES

Dr. C. L. Shear of the Bureau of Plant Industry, Washington, D. C., is spending a good part of the winter making a mycological and pathological survey of the Hawaiian Islands and expects to bring back a large collection of fungi for study.

Dr. Frederick A. Wolf, Pathologist, Fruit Disease Investigations, United States Department of Agriculture, for the past two years stationed at Orlando, Florida, has accepted a position at Duke University, Durham, North Carolina.

Dr. Jaroslaw Peklo, Professor of Applied Botany and Phytopathology at the Check Technical University, Prague, has come to the United States in order to pursue his studies in mycology, phytopathology and breeding of plants resistant to disease. He will be in this country about nine months and is at present at the Bureau of Plant Industry, Washington, D. C.

"Die Pilze Mitteleuropas," edited by H. Kniep, P. Claussen, and J. Basz, published by Dr. Werner Klinkhardt, Leipzig, 1927. Price 5 Marks per Lieferung.

Three folio parts of this illustrated work on the fleshy fungi of Central Europe have already appeared. The fungi are fully described and illustrated in colors from photographs taken from fresh specimens. The illustrations are of unusual excellence and are not confined to single specimens but include a number of individuals of different ages and conditions. Detailed drawings of basidia, cystidia and spores are also given. The three parts which have already appeared are devoted to species of *Boletus*. It is intended to publish the parts at intervals of two or three months. This work ought to be of great usefulness to all mycologists, mushroom collectors and others interested in fungi. C. L. SHEAR.

In the second paper on "Additions to the Australian Ascomycetes," Dr. Ethel McLennan and Isabel Cookson, of the

University of Melbourne, have reported three species of *Lamprospora* which were collected and named by Dr. Fred J. Seaver from material collected in and about The New York Botanical Garden. The three species are as follows: *Lamprospora tuberculata* Seaver; *Lamprospora tuberculatella* Seaver; *Lamprospora Maireana* Seaver. An Australian variety of a fourth species has been established, *Lamprospora areolata* Seaver, var. *australis* McLennan and Cookson. Two of the above named species are known only in New York and Australia. Misses McLennan and Cookson indicate their intention of continuing their survey of the Discomycetes of Australia and it is very interesting to note the cosmopolitan habits of some of these fungi.

FIFTH INTERNATIONAL BOTANICAL CONGRESS, CAMBRIDGE, 1930

At the International Congress of Plant Sciences (Fourth International Botanical Congress) held at Ithaca, United States, in August, 1926, an invitation was conveyed from British Botanists for the Fifth International Botanical Congress to be held in England in 1930. The invitation was accepted by the Botanists assembled at Ithaca, and arrangements are now being made for the Congress to be held at Cambridge about the middle of August, 1930.

An Executive Committee has been formed to make arrangements for the Congress, consisting of Dr. F. F. Blackman, Prof. V. H. Blackman, Dr. E. J. Butler, Prof. Sir John Farmer, Prof. F. E. Fritsch, Prof. Dame Helen Gwynne-Vaughan, Dr. A. W. Hill, Prof. W. Neilson Jones, Sir David Prain, Dr. A. B. Rendle (Treasurer), Prof. A. C. Seward (Chairman), Prof. W. Stiles, and Prof. A. G. Tansley.

It has been decided to organize the Congress in the following seven sections: Morphology (including Anatomy), Palaeobotany, Plant Geography and Ecology, Taxonomy and Nomenclature, Genetics and Cytology, Physiology, and Mycology and Plant Pathology.

Mr. F. T. Brooks, The Botany School, University of Cambridge, England, and Dr. T. F. Chipp, Royal Botanic Gardens, Kew, England, have been appointed Honorary Secretaries of the Congress, and any communications with regard to the Congress should be addressed to one or other of the Secretaries.